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	04151862001 Patents ADP number *(if you know it)*	
	If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom
4.	Title of the invention	"Bacterial Supplement"
5.	Name of your agent *(if you have one)*	Murgitroyd & Company
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Scotland House 165-169 Scotland Street Glasgow G5 8PL
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Murgitroyd & Co

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Date: 26 March 2003

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Figure 1

Sequence information for five of the lactate utilising strains.

S D6 1L/1

GATGAACGCTGGCGGCGTGCCTAACACTGCAAGTCGAACGAAGCACCTTACCTGATTCTTCGGAT
GAAGGTCTGGTGACTGAGTGGCGGACGGGTGAGTAACGCGTGGGTAACCTGCCCTGTACAGGGGG
ATAACAGTTGGAAACGGCTGCTAATACCGCATAAGCGCACGAGAGGACATCCTCTTGTGTGAAAA
ACTCCGGTGGTACAGGATGGGCCCGCGTCTGATTAGCTGGTTGGCAGGGTAACGGCCTACCAAGG
CGACGATCAGTAGCCGGTCTGAGAGGATGAACGGCCACATTGGAACTGAGACACGGTCCAACTCA
TACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCAACGCCGCGTGA
GTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGGGAAGATAATGACGGTACCTGACTAAG
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ACTGGGTGTAAAGGGTGCGTAGGTGGCAGTGCAAGTCAGATGTGAAAGGCCGGGGCTCAACCCCG
GAGCTGCATTTGAAACTGCATAGCTAGAGTACAGGAGAGGCAGGCGGAATTCCTAGTGTAGCGGT
GAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCCTGCTGGACTGTTACTGACACT
GAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGA
ATACTAGGTGTCGGGGCCGTATAGGCTTCGGTGCCGTCGCAAACGCAGTAAGTATTCCACCTGGG
GAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATG
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AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATCTTCAGTAGCCAGCAGGTAAGGCTG
GGCACTCTGGAGAGACTGCCAGGGATAACCTGGAGGAAGGTGGGGACGACGTCAAATCATCATG
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GGGGAGCAAACCACAAAAAGGCCGTCCCAGTTCGGACTGTAGTCTGCAACCCGACTACACGAAG
CTGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACA
CCGCCCGTCACACCATGGGAGTCGGAAATGCCCGAAGCCAGTGACCCAACCATATGGAGGGAGC
TGTCGAAGGTGGAGCCGGTAACTGGGGTG

S 6M/1

GATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGAAGCACCTTACGAGATTCTTCGGA
TGATCGTTTGGTGACTGAGTGGCGGACGGGTGAGTAACGCGTGGGTAACCTGCCCTGTACAGGGG
GATAACAGCTGGAAACGGCTGCTAATACCGCATAAGCGCACGAGGAGACATCTCCTAGTGTGAAA
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GCAACGATCAGTAGCCGGTCTGAGAGGATGAACGGCCACATTGGAACTGAGACACGGTCCAAACT
CCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCAACGCCGCGT
GAGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGGGAAGATAATGACGGTACCTGACTA
AGAAGCTCCGGCTAAATACGTGCCAGCAGCCGCGGTAATAGATATGGAGCAAGCGTTATCCGGAT

TTACTGGGTGTAAAGGGTGCGTAGGTGGCAGTGCAAGTCAGATGTGAAAGGCCGGGGCTCAACCC
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CTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGAT
GAATACTAGGTGTCGGGGCCGTATAGGCTCCGGTGCCGCCGCTAACGCAGTAAGTATTCCACCTG
GGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCAT
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GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATCTTCAGTAGCCAGCAGGTAAGGCTGGG
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CTTATGATCTGGGCGACACACGTGCTACAATGGCGGTCACAGAGTGAGGCGAACCCGCGAGGGGG
AGCAAACCACAAAAAGGCCGTCCCAGTnCGGACTGTAGTCTGCAACCCGACTACACAGAAGCTGG
AATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCC
CGTCACACCATGGGAGTCGGAAATGCCCGAAGCCAGTGACCCAACCTTTATGAAGGAAGCCnGTC
CAAGGTTGAACCCGTTAACTGGGGnnTT

Ss3/4

GAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGAGGT
ATATTGAATTGAAGTTTTCGGATGGATTTCAATGATACCGAGTGGCGGACGGGTGAGTAACGCGT
GGGTAACCTGCCTCATACAGGGGGATAACGGTTAGAAATGACTGCTAATACCGCATAAGCGCACA
GTACCGCATGGTACGGTGTGAAAAACTCCGGTGGTATGAGATGGACCCGCGTCTGATTAGCTAG
TTGGTGGGGTAACGGCCCACCAAGGCGACGATCAGTAGCCGACCTGAGAGGGTGACCGGCCACA
TTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGGATATTGCACAATGGAG
GAAACTCTGATGCAGCGACGCCGCGTGAGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGC
AGGGAAGAAAATGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTA
ATACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAGACGGCGACGCAA
GTCTGAAGTGAAATACCCGGGCTCAACCTGGGAACTGCTTTGGAAACTGTGTTGCTAGAGTGCT
GGAGAGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAAGAACACCAGTG
GCGAAGGCGGCTTACTGGACAGTAACTGACGTTGAGGCTCGAAAGCGTGGGGAGCAAACAGGAT
TAGATACCCTGGTAGTCCACGCCGTAAACGATGAATACTAGGTGTTGGTGAGCAAAGCTCATCG
GTGCCGCCGCAAACGCAATAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAG
GAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
CTTACCAAATCTTGACATCCCTCTGAAAARYCCYTTAATCGGRTTCCTCCTTCGGGACAGAGGT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCCTATTGTCAGTAGCCAGCAGGTGAAGCTGGGCACTCTGATGAGACTGCCAGGGATA
ACCTGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTG
CTACAATGGCGTAAACAAAGAGAAGCGAGCCTGCGAGGGGGAGCAAATCTCAAAAATAACGTCT
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AATGCTGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCGGAA

ATGCCCGAAGCCAGTGAACCCAATGCGAAAGCAGGGAGCTGTCGAAGGCAGGTCTGATAACTGGG
GTG

Ss2/1 and Ssc/2

AGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGAAA
CACCTTATTTGATTTTCTTCGGAACTGAAGATTTGGTGATTGAGTGGCGGACGGGTGAGTAACG
CGTGGGTAACCTGCCCTGTACAGGGGGATAACAGTCAGAAATGACTGCTAATACCGCATAAGAC
CACAGCACCGCATGGTGCAGGGGTAAAAACTCCGGTGGTACAGGATGGACCCGCGTCTGATTAG
CTGGTTGGTGAGGTAACGGCTCACCAAGGCGACGATCAGTAGCCGGCTTGAGAGAGTGAACGGC
CACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT
GGGGGAAACCCTGATGCAGCGACGCCGCGTGAGTGAAGAAGTATCTCGGTATGTAAAGCTCTAT
CAGCAGGGAAGAAAATGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGC
GGTAATACGTAGGGGGCAAGCGTTATCCGGAATTACTGGGTGTAAAGGGTGCGTAGGTGGTATG
GCAAGTCAGAAGTGAAAACCCAGGGCTTAACTCTGGGACTGCTTTTGAAACTGTCAGACTGGAG
TGCAGGAGAGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACATC
AGTGGCGAAGGCGGCTTACTGGACTGAAACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACA
GGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATACTAGGTGTCGGGGCCGTAGAGGC
TTCGGTGCCGCAGCCAACGCAGTAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAACTCA
AAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGA
ACCTTACCTGGTCTTGACATCCTTCTGACCGGTCCTTAACCGGACCTTTCCTTCGGGACAGGAG
TGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCCTATCTTTAGTAGCCAGCATATAAGGTGGGCACTCTAGAGAGACTGCCAGGGATA
ACCTGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTATGACCAGGGCTACACACGTG
CTACAATGGCGTAAACAGAGGGAAGCAGCCTCGTGAGAGTGAGCAAATCCCAAAAATAACGTCT
CAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGCGAATCAG
AATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCAGTA
ACGCCCGAAGTCAGTGACCCAACCGTAAGGAGGAGCTGCCGAAGCGGACCGATAACTGGGGTG
AAGTCGTAACCAGGTAGCCGT

W = A or T
Y = T or C
R = G or A
N = Unknown

Bacterial Supplement

This invention relates to improvements in health and nutrition for both animals and humans following the ingestion of specific bacteria capable of utilising lactic acid.

Under normal conditions the concentration of lactic acid (lactate) in the mammalian gut is very low despite the fact that many bacterial species, such as lactobacilli, streptococci, enterococci and bifidobacteria that reside in the intestine produce this acid in large quantities as a fermentation end product. Lactic acid is also produced by host tissues.

It has been hypothesised that the accumulation of lactic acid is normally prevented by the ability of certain other bacteria that inhabit the gut to consume lactic acid and to use it as a source of energy. The identity of the microorganisms that are postulated to conduct this metabolic process in the mammalian large intestine has largely not previously

been elucidated, Bourriaud et al (2002). Kanauchi et al (1999) revealed that a strain of *Bifidobacterium longum* was co-incubated with a strain of *Eubacterium limosum* on germinated barley feedstuff for three days there was a marked increase in acetate formed and a small increase (less than 3 mM) in butyrate formed when compared to the incubations with *E. limosum* alone.

In the rumen of cattle and sheep the species *Selenomonas ruminantium, Veillonella parvula* and *Megasphaera elsdenii* are regarded as the most numerous utilisers of lactate (Gilmour *et al.*, 1994; Wiryawan and Brooker, 1995). The contribution of *Megasphaera elsdenii* appears to be particularly significant in the rumen, based on the high proportion of carbon flow from lactic acid to propionic acid and this species employs the acrylate pathway for this purpose (Counotte *et al.*, 1981). *Megasphaera elsdenii* produces a variety of end products including propionate, butyrate, caproate and branched chain fatty acids from lactate (Ushida et al (2002), Kung and Hession, (1995)). This probably reflects the ability of this species to use lactate despite the presence of other carbon sources such as sugars, whereas *Selenomonas* uses lactic acid only in the absence of other energy sources. This has led to interest in the use of *Megasphaera* as a probiotic organism that might be added to animal (Kung and Hession, 1995; Ouwerkerk *et al.*, 2002), or even human diets to prevent the harmful accumulation of lactic acid. In ruminant animals (cattle and sheep)

accumulation of lactic acid occurs when a large amount of readily fermentable substrate (such as starch and sugars) enters the rumen. Rapid fermentation, particularly by organisms such as *Streptococcus bovis*, drives down the pH, creating more favourable conditions for the proliferation of lactic acid producing bacteria such as lactobacilli, and *S. bovis* itself. Normal populations of bacteria capable of utilising lactate (lactate utilisers) are unable to cope with the greatly increased production of lactic acid. Unaided, lactic acid may accumulate to levels that can cause acute toxicity, laminitis and death (Nocek, 1997; Russell and Rychlik, 2001).

Similar events occurring in the large intestine can also cause severe digestive and health problems in other animals, for example in the horse where high lactate levels and colic can result from feeding certain diets.

In humans lactic acid accumulation is associated with surgical removal of portions of the small and large intestine, and with gut disorders such as ulcerative colitis and short bowel syndrome (Day and Abbott, 1999). High concentrations of lactic acid in the bloodstream can cause toxicity (Hove *et al.*, 1994), including neurological symptoms (Chan *et al.*, 1994). Much of this lactic acid is assumed to derive from bacterial fermentation, particularly by bifidobacteria and by lactobacilli and enterococci. Lactic acid can also be produced by host tissues, but

the relative contributions of bacterial and host sources are at present unclear.

Conversely, the formation of other acid products, in particular butyric acid (butyrate), is considered to be beneficial as butyric acid provides a preferred energy source for the cells lining the large intestine and has anti-inflammatory effects (Inan *et al.*, 2001, Pryde *et al.*, 2002). Butyrate also helps to protect against colorectal cancer and colitis (Archer *et al.*, 1998; Csordas, 1996).

We have now established a method of isolating novel bacteria that are remarkably active in consuming lactic acid from human faeces. Preferably the method allows isolation of bacteria which convert the lactic acid to butyric acid. According to this method several new bacteria that are remarkably active in converting lactic acid to butyric acid have been isolated.

One group of these bacteria is from the newly described genus *Anaerostipes caccae* (Schwiertz *et al.*, 2002). Although some main characteristics of *A. caccae* are described in this publication, its ability to use lactate was not reported and has only recently been recognised as described herein.

The invention relates to a method for selecting a strain of lactic acid-utilising bacteria, which method comprises the steps of:

a) Providing (for example isolating) a bacterial culture from a human faecal sample;

b) selecting a single colony of bacteria;

c) growing said colony in a suitable medium containing lactic acid; and

d) selecting a strain of bacteria consuming relatively large amounts of lactic acid, all of the above steps being conducted under anaerobic conditions.

In the above method, the reference to "relatively large amounts of lactic acid" is defined as meaning the bacteria used more than 10 mM of D, L or DL lactic acid during growth into stationary phase, or 24 hours at 37°C in YCFALG or YCFAL medium.

Preferably the strain of lactic acid utilising bacteria also produces high level of butyric acid and the method of the invention may therefore comprise an additional step of:

e) selecting a strain of bacteria producing relatively large quantities of butyric acid.

In the above step the reference to "relatively large quantities of butyric acid" is defined as meaning the bacteria produces more than 10 mM of butyric acid during growth into stationary phase, or 24 hours at 37°C in YCFALG or YCFAL medium.

Preferably the strain of lactic acid utilising bacterium must be capable of converting lactate produced by another gut bacterium from dietary components such as resistant starch.

Preferably the lactic acid used in step c) is both D- and L- isomers of lactic acid.

Preferably the suitable medium to grow bacteria is nutritionally rich medium in anaerobic Hungate tubes.

Preferably the selected strain of bacteria is re-purified using nutritionally rich medium in anaerobic roll tubes.

A further aspect of the invention is a bacterial strain that produces butyric acid as its sole or predominant fermentation product from lactate and which has been isolated according to the method of the invention described above.

The bacteria *A. caccae* strain L1-92 deposited at NCIMB (National Collections of Industrial, Marine and Food Bacteria in Aberdeen, United Kingdom) under No 13801^T on 4 November 2002 and at DSM under No 14662 on 4 November 2002.

The bacteria strain Ss2/1 *Cl. indolis*-like deposited at NCIMB under No 41156 on 13 February 2003.

The bacteria strain S6M/1 of *Eubacterium hallii* deposited at NCIMB under No. 41155 on 13 February 2003.

Another aspect of the invention is a strain of bacteria having a 16S rRNA gene sequence which differs at less than 3% of residues out of approximately 1400 from one of the sequences shown in Figure 1.

Another aspect of the invention is the use of at least one of the above-mentioned bacterial strains in a medicament or foodstuff.

Another aspect of the invention is a method to promote butyric acid formation in the intestine of a mammal which includes the administration of a therapeutically effective dose of at least one of the above described strains of live butyric acid producing bacteria. The bacterial strain may be administered by means of a foodstuff or suppository or any other suitable method.

Another aspect of the invention is a method for treating diseases associated with a high dosage of lactic acid such as lactic-acidosis, short bowel syndrome and inflammatory bowel disease, including ulcerative colitis and Crohn's disease, which comprises the administration of a therapeutically effective dose of *Anaerostipes caccae* or at least one above-mentioned strains of live lactic acid utilising

bacteria. Advantageously the strain selected may also produce a high level of butyric acid.

Further, another aspect of the invention is a prophylactic method to reduce the incidence or severity of colorectal cancer or colitis in mammals caused in part by high lactic acid and low butyric acid concentrations, which method comprises the administration of a therapeutically effective dose of at least one above identified strains of live lactic acid utilising bacteria and/or butyric acid producing bacteria mentioned above or of *Anaerostipes caccae*.

Another aspect of the invention is the use of live *Anaerostipes caccae* or at least one of the above mentioned lactic acid utilising bacteria as a medicament. Advantageously the strain chosen may produce butyric acid as its sole or predominant fermentation product from lactate. Preferably the bacteria are used in the treatment of diseases associated with high levels of lactic acid such as lactic acidosis, short bowel syndrome and inflammatory bowel disease including ulcerative colitis and Crohn's disease.

According to another aspect of the invention at least one lactate-utilising strain of bacteria as mentioned above or *Anaerostipes caccae* are used in combination with lactic acid producing bacteria including those such as *Lactobacillus* spp. and *Bifidobacterium* spp. or other additives or growth enhancing supplement currently used as probiotics.

The combination of strains would potentially enhance the health-promoting benefits of the lactic acid bacterium by converting its fermentation products (lactic acid alone or lactic acid plus acetic acid) into butyrate. Indeed it is possible that certain health-promoting properties currently ascribed to lactic acid bacteria might actually be due to stimulation of other species such as lactate-consumers *in vivo*, particularly where probiotic approaches (see below) are used to boost native populations in the gut. Furthermore the presence of the lactic acid producing bacteria in a combined inoculum could help to protect the lactate consumer against oxygen prior to ingestion.

The growth and activity of the novel bacteria may be promoted by means of providing certain growth requirements, required for optimal growth and enzyme expression to the bacteria, present in the animal or human gastrointestinal tract. These bacterial growth enhancing nutrients are often referred to as prebiotics or synbiotics.

Thus the invention provides methods to promote the growth and enzyme expression of the microorganism and hence removal of lactate and production of butyrate *in vivo*, for example, via a prebiotic or synbiotic approach (Collins and Gibson, 1999).

Another aspect of the invention is a method for treating acidosis and colic in animals, particularly in ruminants and horses or other farm animals, by

administration of a therapeutically effective dose of *Anaerostipes caccae* or at least one of the lactate utilising bacteria mentioned above. Advantageously the bacteria can be administrated as feed additives.

For the use, prevention or treatment of conditions described herein, the bacteria or prebiotic(s) or synbiotic(s) are preferentially delivered to the site of action in the gastro-intestinal tract by oral or rectal administration in any appropriate formulae or carrier or excipient or diluent or stabiliser. Such modes of delivery may be of any formulation included but not limited to solid formulations such as tablets or capsules; liquid solutions such as yoghurts or drinks or suspensions. Ideally, the delivery mechanism delivers the bacteria or prebiotic or synbiotic without harm through the acid environment of the stomach and through the rumen to the site of action within the gastro-intestinal tract.

Another aspect of the invention is the use of at least one bacterial strain mentioned above or *Anaerostipes caccae* in a method to produce butyric acid from lactate and acetate. The method includes the fermentation of the above described microorganism selected for both their lactic acid utilising and butyric acid producing abilities in a medium rich in lactate and acetate. The method can be used in industrial processes for the production of butyrate on a large scale.

Brief description of the Figure

Figure 1

Sequence information for five of the lactate utilising strains.

DETAILED DESCRIPTION

The experimental work performed shows the following:

1. Certain human colonic anaerobic bacteria, including *A. caccae* strains, are strong and efficient utilisers of lactic acid.
2. Certain human colonic anaerobic bacteria, including *A. caccae* strains, are strong and efficient producers of butyric acid.
3. Certain human colonic anaerobic bacteria, including *A. caccae* strains, convert lactic acid to butyric acid.

Example 1: Isolation and characterisation of bacteria

The bacterial strains that were isolated at RRI were selected as single colonies from a nutritionally rich medium in anaerobic roll tubes as described by Barcenilla *et al.* (2000). The isolates were grown in M2GSC broth and the fermentation end products determined. Butyrate producing bacteria were re-purified using roll tubes as described above. Strains L1-92, S D8/3, S D7/11, A2-165, A2-181, A2-183, L2-50 and L2-7 were all isolated using this medium. Omitting rumen fluid and/or replacing the

sugars with one additional carbon source such as DL lactate increased the selectivity of the roll tube medium and this medium was used to isolate strain S D6 1L/1. Strains G 2M/1 and S 6M/1 were isolated from medium where DL-lactate was replaced with mannitol (0.5%). Separately, non-rumen fluid based media routinely used for isolating *Selenomonas* sp., namely Ss and Sr medium (Atlas, 1997) was used to isolate other strains. Inoculating Sr medium roll tubes with dilutions of faecal samples resulted in the isolation of strain Sr1/1 while the Ss medium resulted in the isolation of strains Ss2/1, Ss3/4 and Ssc/2.

Example 2: *A. caccae* and other human colonic bacterial isolates consumes lactic acid and acetic acid and produces butyric acid when grown in rumen fluid

Table 1 summarises the fermentation products formed by twelve strains of anaerobic bacteria when grown under 100% CO_2 in a rumen fluid-containing medium containing 0.5% lactate (M2L) or 0.5% lactate, 0.2% starch, 0.2% cellobiose and 0.2% glucose (M2GSCL) as the energy sources. Ten of these strains were isolated at RRI from human faeces as described above. Strains 2221 and NCIMB8052 are stock collection isolates not from the human gut and are included for comparison. Table 1 demonstrates that three strains, L1-92 (*A. caccae*), SD6 1L/1 and SD 6M/1 (both *E. hallii* -related) all consumed large amounts of lactate (>20mM) on both media examined, M2L and

M2GSCL, and produced large quantities of butyric acid. *A. caccae* L1-92 in particular consumed large amounts of lactate and produced large amounts of butyrate. Acetate is also consumed by all three strains. The other 9 butyrate producing bacteria tested either consumed relatively small amounts of lactate, or consumed no lactate, on this medium.

Table 1. Comparison of human faecal isolates for the ability to utilise (negative values) or produce (positive values) lactate on a rumen fluid based medium (M2) supplemented with lactate (M2L) and lactate plus glucose, cellobiose and soluble starch (0.2% each) (M2GSC).

Table 1

Strain ID	Closest relative	Medium	Formate	Acetate	Butyrate	Lactate
S D8/3	Adhufec 406*+	M2L	1.15	0.97		-3.94
"		M2GSCL	21.66	0.77	10.88	6.43
S D6 1L/1	*E. hallii*	M2L		-19.74	35.48	-32.41
"		M2GSCL		-9.78	22.58	-21.85
S 6M/1	*E. hallii*	M2L	0.79	-19.01	31.73	-23.72
"		M2GSCL	1.31	-5.06	22.77	-28.42
G 2M/1	HucA19*	M2L		2.82	7.97	23.66
"		M2GSCL		0.01	12.94	9.52
S D7 11/1	ND#	M2L		0.51	0.08	7.58
"		M2GSCL	1.85	0.43	4.84	-10.25
2221	*But. Fibrisolvens*	M2L	1.37	-3.61	1.57	21.57
"		M2GSCL	19.4	-5.57	18.02	11.75
8052	*Cl. acetobutylicum*	M2L		-12.42	19.31	-0.87
"		M2GSCL	0.13	-1.79	18.00	-5.80
A2-165	*F. prausnitzii*	M2L	1.98	0.62	3.56	2.94
"		M2GSCL	17.47	-6.97	18.38	-5.65

Strain ID	Closest relative	Medium	Formate	Acetate	Butyrate	Lactate
A2-183	*Roseburia* sp.	M2L		0.86	1.84	10.63
"		M2GSCL	-0.15	-12.70	18.23	5.33
A2-181	*Roseburia* sp.	M2L	0.58	-0.26	1.75	
"		M2GSCL	0.33	-11.05	18.68	5.22
L2-50	*Coprococcus* sp.	M2L	1.06	2.32	0.52	0.43
"		M2GSCL	19.37	4.47	7.60	3.41
L1-92	*Anaerostipes caccae*	M2L		-29.42	37.00	-25.60
"		M2GSCL	0.63	-27.03	44.78	-45.48

* clone library sequence, uncultured (Hold *et al.*, 2002)

+ clone library sequence, uncultured (Suau *et al.*, 1999)

ND not determined

Example 3: *A. caccae* and other human colonic bacterial isolates consumes lactic acid and acetic acid and produces butyric acid when grown in rumen fluid free medium

Table 2 (a) shows the utilisation and production of formate, acetate, butyrate, succinate and lactate, on this occasion performed using the rumen fluid-free medium YCFA (Duncan *et al.* 2002) containing no added energy source, or with 32 mM lactate (YCFAL) or lactate plus 23 mM glucose (YCFALG) as added energy sources. Separately table 2 (b) reveals the levels of the two isomers of lactate (D and L) remaining at the end of the incubations and the concentration of glucose metabolised during the incubations. Five additional new lactate-utilising isolates were discovered using the semi-selective medium as described earlier and are included in Tables 2 (a) and (b), although one of these (Ss 3/4) proved to consume a relatively small amount of lactate only on the YCFAL medium (Table 2a). Analysis of the consumption of the D and L isomers reveals that three strains (Ss2/1, Ssc/2 and Sr1/1) preferentially consumed D lactate. Partial repression of lactate consumption by glucose was observed on this medium with *A. caccae* L1-92, and almost complete repression for SD D6 1L/1 and Ss 3/4. The previously isolated *E. hallii* strain L2-7 (Barcenilla *et al.*, 2000) behaved in a similar manner to SD D6 1L/1. The higher glucose concentration in this medium compared with M2GSCL is likely to explain the difference in behaviour of *A. caccae* compared with Table 1. The

remaining five strains showed no evidence of repression of lactate utilisation in the presence of glucose although it is possible they use the glucose before switching to lactate. Butyrate levels exceeding 30mM were obtained for four strains on YCFALG medium.

Table 2a. Fermentation products formed or utilised (U as indicated by minus values) by human gut isolates incubated on yeast extract-casitone-fatty acids medium (YCFA); YCFA supplemented with lactate (YCFAL); and YCFA supplemented with glucose and lactate (YCFALG). The initial concentration of glucose added to the medium was 23 mM and 32 mM lactate was added that contained 15.5 mM L-lactate. [a] Strain identity is based on 16S rRNA sequence information (% identical residues with closest relative is shown). See appendix 1 for sequence information.

All strains except 2221 and 8052 (Table 1) isolated at RRI.

Table 2a

Strain ID	Closest relative[a]	Isolation Medium	Medium	Formate	Acetate P/U	Butyrate	Succin	Lactate P/U
Ss2/1	*Cl. indolis* (95%)	Selenomonas selective	YCFA	0.02±0.04	-4.25±4.68	2.24±0.26		0.39±0.03
			YCFAL	0.18±0.02	-12.51±1.27	12.98±0.19		-15.27±2.53
			YCFALG	10.10±1.05	-24.32±1.03	35.69±1.13		-13.95±2.70
Sr 1/1	HucB 12*	Selenomonas ruminantium	YCFA		-5.42±1.77	2.33±0.03		0.36±0.12
			YCFAL	0.76±0.19	-13.35±2.27	14.15±0.17		-15.04±0.89
			YCFALG	9.53±2.03	-22.47±1.40	35.77±1.50		-13.71±0.40
S D6 1L/1	*E. hallii* HucA 15*	M2 + 0.5% lactate	YCFA		-4.96±3.26	1.42±0.23		
			YCFAL		-18.51±0.96	21.06±1.06		-29.93±0.60
			YCFALG		-9.22±2.52	20.78±1.52		-2.43±0.70
S 6M/1	*E. hallii* (98%)	M2 + 0.5% mannitol	YCFA	0.09±0.03	-2.61±2.36	1.42±0.05		
			YCFAL	0.21±0.1	-7.20±2.08	6.54±0.43		-6.27±1.27
			YCFALG	20.68±	-10.95±	29.2±		-25.82±

Strain ID	Closest relative[a]	Isolation Medium	Medium	Formate	Acetate P/U	Butyrate	Succin	Lactate P/U
Ss 3/4	HucA19* (New species to be named)	Selenomonas selective	YCFA		4.75±2.20	6.10±0.27		1.09±0.47
			YCFAL		6.68±2.09	6.19±0.34		-9.78±2.56
			YCFALG	0.54±0.13	5.06±4.28	8.66±0.53		3.86±1.09
Ssc/2	*Cl. indolis* (95%)	Selenomonas selective	YCFA	0.25±0.04	-0.16±1.32	2.37±0.09		0.48±0.03
			YCFAL	0.36	-12.12	13.49		-13.78
			YCFALG	10.98±1.27	-25.35±2.87	36.10±0.49		-13.34±1.28
L1-92	*A. caccae (type strain)*	M2GSC	YCFA	0.00±0.08	-2.35±2.03	1.99±0.09		
			YCFAL	-0.05±0.10	-21.98±2.45	23.35±1.16		-28.92±0.54
			YCFALG	1.49±0.13	-26.83±0.58	36.81±3.61		-12.01±1.32
L2-7	*E. hallii*	M2GSC	YCFA	0.02±0.01	-1.58±1.73	0.63±0.03		0.00±0.00
			YCFAL	1.09±1.55	-14.77±0.93	22.58±0.76		-30.47±0.00
			YCFALG	3.93±3.38	12.78±0.94	5.80±0.97		1.67±0.47

* clone library sequences, uncultured (Hold *et al.*, 2002)

Table 2b. Total lactate (mM) remaining in the tubes at the end of the 24 h incubation period and separately the concentration of the two forms D and L. Total glucose (gluc) metabolised during growth also recorded (mM).

Strain number	Closest relative	Medium	Total lact.	L-lact	D-lact	Gluc used
Ss2/1	*Cl. indolis* (95%)	YCFA	0.84±0.02			
		YCFAL	17.08±2.53	16.07±0.40	1.01±2.15	
		YCFALG	18.40±2.70	15.90±1.06	2.50±3.30	22.1±0.0
Sr 1/1	Huc B12*	YCFA	0.81±0.12			
		YCFAL	17.31±0.89	15.05±0.34	2.26±0.68	
		YCFALG	18.64±0.40	16.37±0.79	2.27±0.71	22.0±0.2
S D6 1L/1	*E. hallii* Huc A15*	YCFA	0.00±0.00			
		YCFAL	2.42±0.60	0.21±0.10	2.21±0.51	
		YCFALG	29.92±0.07	10.65±0.69	19.27±0.79	22.1±0.1
S 6M/1	*E. hallii* (98%)	YCFA	0.00±0.00			
		YCFAL	26.08±1.27	9.94±0.50	16.14±1.06	

Strain number	Closest relative	Medium	Total lact.	L-lact	D-lact	Gluc used
		YCFALG	6.57±0.16	4.02±2.26	2.55±2.32	22.1±0.1
Ss 3/4	HucA19* (new species to be named)	YCFA	1.54±0.47			
		YCFAL	22.58±2.55	16.56±0.12	6.02±2.65	
		YCFALG	36.21±1.09	16.95±0.87	19.26±1.91	16.6±0.6
Ssc/2	*A. caccae* (L1-92)	YCFA	0.96±0.08			
		YCFAL	22.39±6.63	15.40±0.78	6.99±6.10	
		YCFALG	19.01±1.28	15.08±0.93	3.93±0.68	22.2±0.0
L1-92	*A. caccae* (type strain)	YCFA	0.0±0.0			
		YCFAL	3.43±0.54	1.84±0.85	1.59±0.87	
		YCFALG	20.34±1.32	8.63±0.72	11.71±2.01	
L2-7	*E. hallii*	YCFA	0.00±0.00			
		YCFAL	0.00±0.00			
		YCFALG	31.93±0.47	15.43±0.12	16.50±0.30	11.99±0.71

* clone library sequence, uncultured (Hold *et al.*, 2002)

Table 3a. Fermentation profiles for *Bifidobacterium adolescentis* L2-32 and three lactate utilisers when incubated alone or in co-culture for 24 hours at 37°C on modified YCFA medium (modified to contain 0.1% casitone) containing 0.2% soluble starch.

Culture/ co-culture	Formate	Acetate	Butyrate	Total Lactate	L-Lactate
L2-32	4.29±0.92	51.04±5.44	0	5.00±0.09	5.16±0.45
L1-92	0.01±0.01	34.99±0.93	1.57±0.26	0.40±0.69	0
SD6M/1	0	35.25±2.15	0.75±0.06	0.27±0.27	0
L2-7	0.04±0.06	35.70±0.44	0.83±0.02	0	0
L2-32+L1-92	4.29±0.04	44.82±1.13	7.62±0.66	0.61±0.53	0
L2-32+SD6M/1	4.81±1.08	48.17±6.47	6.23±1.15	0	0
L2-32+L2-7	5.16±1.37	43.88±3.74	7.35±0.27	0.36±0.01	0

Table 3b. Total viable counts (cfu per ml) of *Bifidobacterium adolescentis* L2-32 and three ~~lactate utilisers following 24 hours at 37°C in~~ monoculture and co-culture. *Bifidobacterium adolescentis* L2-32 was selected for on MRS + 0.25% propionate roll tubes and the butyrate producing/lactate utilisers were selected for on M2 + 0.5% lactate roll tubes following incubation for 24 hours at 37°C.

Culture / Co-culture	*B. adolescentis* L2-32	Butyrate producer / lactate utiliser
L2-32	3.8×10^8	
L1-92		2.4×10^8
SD6M/1		1.0×10^7
L2-7		8.0×10^6
L2-32+L1-92	6.4×10^8	1.7×10^9
L2-32+SD6M/1	3.8×10^8	6.8×10^8
L2-32+L2-7	3.2×10^8	5.4×10^9

Summary

A. caccae strain L1-92 was able to consume up to 30mM DL lactate, along with 20-30 mM acetate during batch culture incubation for 24 hours at 37°C with the production of >20mM, and up to 45mM butyrate; this occurred also when glucose was added as an alternative energy source (Table 1). Lactate or lactate plus glucose thus resulted in very much higher production of butyrate than observed with 23mM glucose alone, when only <15mM butyrate was formed. Furthermore none of the 74 strains

screened previously by Barcenilla *et al.* (2000) produced more than 25mM butyrate when tested in M2GSC medium. Lactate consumption is not a general characteristic of butyrate-producers, and six of the strains screened in Table 1 failed to consume lactate in M2GSCL medium.

Six further strains that are highly active lactate utilisers (defined for example as net consumption of at least 10mM of lactate during growth to stationary phase or for 24 hours in YCFALG or YCFAL medium at 37°C - see Table 2) were obtained following deliberate screening of new human faecal isolates for lactate utilisation. At least two of these (SD6 1L/1 and S6M/1 - Tables 1, 2) are related to *Eubacterium hallii.* (Table 2a), based on determination of their 16S rDNA sequences. These isolates again consume large quantities of lactate and produce high levels of butyrate *in vitro*. With one exception where considerable glucose repression occurred (strain SD6 1L/1), significant lactate utilization occurred in the presence of glucose (Table 2). Three strains (Ss 2/1, Sr 1/1 and Ssc/2) showed preferential utilization of D-lactate, whereas the two *E. hallii*-related strains SD 6M/1, SD6 1L/1 and *A. caccae* L1-92 utilise both isomers (Table 2b). The two stereoisomers differ in their toxicity in the human body, with the D-isomer being regarded as the more toxic (Chan *et al.*, 1994, Hove *et al.*, 1995). The present invention thus provides a means of utilising both D

and L lactate isomers or preferentially utilising D-lactate in preference to L-lactate.

A. caccae and newly isolated bacteria related to *E. hallii* and *Cl. indolis* were shown to consume up to 30mM DL, D or L lactate, along with 20-30 mM acetate during batch culture incubation and convert this energy in to production of at least 20mM, and up to 45mM butyrate. Furthermore, these strains were shown to convert all of the L-lactate produced by a starch-degrading strain of *Bifidobacterium adolescentis* into butyrate when grown in culture. This is the first documentation demonstrating the conversion of lactate to butyrate by human colonic bacteria, some of which are likely to be new species.

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